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BOTANICAL GAZETTE

APRIL, 1903

OOGENESIS IN SAPROLEGNIA.¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY.
XLVI.

BRADLEY MOORE DAVIS.

(WITH PLATES IX AND X)

ALTHOUGH *Saprolegnia* is a form of considerable interest in connection with the problem of the so-called multinucleate gametes, nevertheless investigations have not been carried forward upon it with that attention to cytological detail that has recently been given to other Phycomycetes, *e. g.*, *Albugo*, *Peronospora*, *Pythium*, and *Sclerospora*.

The present paper deals chiefly with the events of oogenesis and a comparison of this process with the development of zoospores. The material employed was apogamous, indeed apandrous, for specimens were chosen entirely free from antheridia to the end that the investigation might be relieved from the dispute on the sexuality of these fungi. However, as will be seen, the results have an important bearing on the well-known binucleate eggs, assumed by Trow to be stages of fertilization. At the end of the paper will be found an account, entitled "Theoretical Considerations," which deals with a number of topics suggested by this study in relation to recent investigations upon Phycomycetes and Ascomycetes.

The material was isolated in pure cultures and cultivated for several months on various substrata, during which time the writer had the opportunity of observing and confirming many of

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the adaptations recorded by Klebs (1899) in his detailed study of *Saprolegnia mixta*. In this period a number of structural peculiarities appeared, associated with the various sorts of nutrition, and forms arose presenting the characters of three closely related species, *Saprolegnia mixta*, *S. monoica*, and *S. ferax*. The variation was most marked in respect to the presence, absence, or relative quantity of antheridia which are the most important distinguishing marks of these species.

The original collection bore oogonia with relatively few antheridia (*Saprolegnia mixta*), and frequently none. By cultivating the form on a rich substratum—raw beef or fresh insects—a much more extensive growth of antheridial filaments was obtained, as in *Saprolegnia monoica*. On other media—boiled whites and yolks of eggs and dried beef—the filaments never produced antheridia, but oogonia were formed abundantly (as in *Saprolegnia ferax*), normal in size and with numerous oospores. After three months all cultures ceased to develop antheridia and the number of oogonia steadily decreased until the cultures reproduced entirely by zoospores. It was always possible to get oospores, as Klebs (1899) has shown, by placing cultures developing zoosporangia under such conditions that the hyphae were no longer submerged. This may readily be done by removing material from water and placing it in a dish of cold agar-agar, which will furnish enough moisture to support the fungus for several weeks. The filaments out of water promptly developed oogonia, even when they had the form characteristic of zoosporangia. Such cultures frequently showed club-shaped oogonia whose eggs were arranged approximately in a line.

Chromacetic acid proved to be the most satisfactory fixing agent, but it must be employed much weaker than the usual formula. One per cent. chromacetic acid caused immediate contraction of the protoplasm, but a solution one-fourth per cent. chromic and one-tenth per cent. acetic acid gave excellent results, and presented advantages of clearness and preservation over weak Flemming, Merkel, corrosive sublimate, sublimate acetic, iridium chlorid, or picric acid. Paraffin sections were cut 3–5 μ thick, and generally stained with safranin and gentian

violet. The protoplasmic structures are so minute as to require lenses of the clearest definition, and the Zeiss apochromatic objectives 2^{mm} and 1.5^{mm} with the compensating oculars were employed throughout the investigation.

OOGENESIS.

The accounts of nuclear and cytoplasmic activities in *Saprolegnia* during oogenesis present some striking contradictions, and leave untouched some phases of a detailed but very significant character. Humphrey (1892) was the first author to apply methods of cytological technique, cutting sections in paraffin, and his studies were followed by the investigations of Trow (1895, 1899) and Hartog (1895, 1896, 1899). The last two authors have expressed very divergent views, asserted with a positiveness that invests their discussions with an atmosphere of personal criticism that need not be reviewed in this paper. It is necessary, however, to consider certain conclusions of the earlier authors with which the present writer is not in accord, and it seems best to do this at the outset, leaving the points of agreement with the present investigation to be taken up in their proper connections.

It is well known that the oogonium of the *Saprolegniales* contains many times more nuclei than the number of eggs ultimately formed. Humphrey and Hartog believed that the nuclei fused with one another, thus reducing the sum total until the requisite number was present. Trow stated that the number was diminished through degeneration and digestion until it was so small that each egg took but a single nucleus. The writer has found no evidence of nuclear fusions as reported by Humphrey and Hartog, and in general supports Trow's view of degeneration. However, there seems to be a reason, not known to Trow, for the selection of the fortunate nuclei destined to preside over the eggs, and a large part of this paper will deal with that subject.

It is also well known that the eggs of the *Saprolegniales* are not infrequently binucleate, and sometimes trinucleate. Humphrey and Hartog considered such conditions as merely the final

stages in the process of general nuclear fusion, the last pairings whereby the eggs become uninucleate. Trow has made much of these binucleate eggs, believing the two nuclei to be sexual and one of them introduced by an antheridial filament. He has been bold enough to assert sexuality for four members of the group : *Saprolegnia declina*, *S. mixta*, *Achlya americana*, and *A. americana cambrica*. Nevertheless, Trow presents very little evidence that the so-called "male" nucleus comes from the antheridial tube, or that the latter structure ever opens into the eggs. The writer cannot justify Trow's conclusions in this matter, believing them premature as to evidence and illogical as to probabilities. The present study will attempt to show that binucleate and trinucleate eggs are to be expected under the peculiar conditions governing oogenesis.

With respect to cytological details, investigations scattered over so long a period as twelve years could hardly be expected to agree. Hartog studied from entire mounts, yet was able to count chromosomes and observe nuclear figures. Trow sectioned in paraffin, and was at first (1895) completely deceived as to the interior structure of the nucleus and the number of chromosomes. In his second paper, however, Trow (1899) concedes that the nuclei in the antheridia and oogonia divide mitotically, but his figures are far from clear as to detail. Trow was also mistaken in his interpretation of the nucleolus.

The present study will give a more detailed account of nuclear structure and activities than any previous paper. But the most important contribution relates to certain cytoplasmic manifestations that seem to determine in large part the results of oogenesis. These cytoplasmic activities place the process of oogenesis in *Saprolegnia* in a new light, bringing it into sympathy with conditions in *Albugo*, *Peronospora*, and *Sclerospora*. They are concerned with that cytoplasmic structure termed the coenocentrum.

It is not strange that Humphrey, Hartog, and Trow failed to find the coenocentrum, for its recognition demands exceptionally good fixation and staining. It is probable that Dangeard saw it when he described an oil globule or fatty mass in the cen-

ter of the egg. It seems possible that Trow may have mistaken it at times for a centrally placed nucleus, to which it bears a certain resemblance that might make the two structures indistinguishable in obscurely stained preparations. The coenocentrum does not appear until the processes of oogenesis are well under way. Previous to this period there are nuclear and cytoplasmic activities of considerable import, and they will be considered first.

It is well known that with the flow of the protoplasm into the swollen tip of a hypha there is apparent that peculiar structure of the protoplasm (*fig. 1*), significant of its streaming movement. The nuclei at that time are very small. When the oogonium is cut off by a septum from the hypha that bears it, the protoplasm becomes distributed almost homogeneously through the interior (*fig. 2*). The nuclei then increase in size, and shortly after show most clearly that detail of structure that is to be expected in the resting nucleus. This structure agrees with the accounts of Harper, Wager, Stevens, and myself for the nuclei in other types of fungi, indicating that the conditions among these lower forms are essentially similar to the nuclear structure of higher plants. As is shown in *figs. 3* and *4*, and especially in *fig. 6*, there is a nuclear membrane inclosing a well-differentiated nucleolus, prominent by its size and staining qualities. Much less conspicuous, but readily demonstrated in well-fixed material, is a loose linin network which contains the chromatic material. Trow's description of a central body containing chromatin and nucleolar matter, but "neither a nucleolus nor a chromosome," must have been founded on inferior preparations. There are certainly no complexities in Saprolegnia comparable to the so-called nucleolus of Spirogyra (Mitzkewitsch, 1898, Wisselingh, 1900).

There is one mitosis in the oogonium, but previous to that event a number of vacuoles are developed which generally result in a peripheral arrangement of the protoplasm around a large central space or vacuole containing cell sap. The vacuoles begin to appear immediately after the oogonium is cut off from the parent hypha (*fig. 2*). They grow larger and run together as bubbles do in soapsuds (*fig. 3*), until finally there are one or

perhaps two large vacuoles in the center, and occasionally smaller ones near the edge (*figs. 10, 11*). The protoplasm then lies as a thick peripheral zone, and the nuclei (*fig. 5*) are distributed at varying distances between the oogonial wall and the boundary of the vacuole.

This is the period when one may expect to find the nuclei in mitosis. This event happens to most nuclei at about the same time, and good preparations of this stage of oogenesis are very striking (*fig. 5*). The oogonium is filled with the diamond-shaped spindles inclosed in nuclear membranes. Three stages of mitosis are shown in *figs. 7-9*. It will be noted that the spindle is intranuclear. *Fig. 7* presents the condition just previous to metaphase, with the chromosomes, four in number, at the nuclear plate and the nucleolus lying outside of the spindle. *Fig. 8* is of a stage shortly after metaphase, when the two sets of daughter chromosomes have separated and are about to pass to the poles; the nucleolus is still present, but smaller and staining faintly. *Fig. 9* is of anaphase, the two groups of daughter chromosomes, four in each, lying at the poles of the spindle and the nuclear membrane manifestly about to disappear. The nucleolus probably dissolves; at least I have never been able to follow it much beyond metaphase, but surviving it would of course soon be lost in the granular cytoplasm after the breaking down of the nuclear membrane. Although granules are sometimes present at the poles of the spindles, the latter are generally entirely free from appearances that might suggest centrosomes.

It will be noted that this description of mitosis in *Saprolegnia* is similar in all essentials to the accounts of Wager (1896), Stevens (1899, 1901), and myself (1900) for *Albugo*; Wager (1900) for *Peronospora*; Miyake (1901) and Trow (1901) for *Pythium*; and Stevens (1902) for *Sclerospora*. The studies cover a wide range of forms and material. They agree in describing the spindle as always intranuclear and without centrosomes. The nucleolus is a structure always distinct from chromatic material and always, so far as we know, disappearing during mitosis by dissolution or extrusion into the cytoplasm. The chromosomes are derived from a linin network, and after mitosis

the chromatin returns to the granular condition generally present in resting nuclei.

Following mitosis, the oogonium passes into a condition that is exceedingly difficult to study. The number of nuclei has been doubled by the division, but the daughter nuclei are much smaller than the parents. A comparison of *fig. 4* with *fig. 10* will illustrate well the change. It is not the small size, however, that makes the examination so difficult, but the fact that these nuclei very shortly show signs of degeneration. Almost all of the nuclei are affected. The nuclear membrane becomes indistinct, and its contents finally lie as granular matter in a clear area that resembles, and probably is, a vacuole. The granular matter is undoubtedly derived in large part from the nucleolus that fragments, but some of it may be chromatin. The study of the steps in this process of general degeneration is especially baffling because the progress is toward a time when the nuclear material becomes indistinguishable from other granules in the cytoplasm.

It is difficult to understand how Humphrey and Hartog could ever have interpreted this process of degeneration as successive nuclear fusions. As Trow pointed out, successive fusions should give more and more conspicuous nuclei, as the material accumulated with each union, and consequently an ever-increasing clearness of conditions. In reality, however, we pass from the stage illustrated by *fig. 10* just after mitosis, to the vague conditions presented in *figs. 11, 14, and 15*. The last two figures are of oogonia much older than those shown in *figs. 10 and 11*, and illustrate late stages in the process, when the nuclear membranes have mostly disappeared and the nucleoli and possibly chromatic material lie in vacuoles. Such vacuoles are frequently elongated, and when they contain two masses of deeply staining material there is suggested a stage in nuclear fusion, and such appearances probably deceived Humphrey and Hartog. However, the vagueness of structure and manifest waning of the previous clear definition should have put these observers on their guard. These degenerate nuclei remain for a long time, even after the eggs are fully formed, and it is quite impossible to tell with exactness when they lose their structure and functions.

The eggs are formed during the process of nuclear degeneration described above, and their nuclear structure is really determined by that event. Trow (1899) has given a very good account of the general stages in this process of protoplasmic segmentation, but he did not know the cytological details, and there is reason to believe that he may have been mistaken in his interpretation of certain structures which he considered nuclei. The first external indication of protoplasmic segmentation is the gathering of the contents of the oogonium into denser masses around certain centers, these masses projecting into the central vacuole and destroying that even outline present in earlier conditions of the oogonium (*fig. 5*). The protoplasm between the egg origins is less dense, and presently begins to develop small vacuoles (*fig. 12*), which run together until the egg origins are separated by spaces of considerable size (*fig. 13*). Many of these vacuoles break through the films of protoplasm into the central space, which then appears to have put out extensions toward the cell wall. The protoplasm of the oogonium is exceptionally mobile at this time, and the vacuoles are constantly changing their form and position. In the end the protoplasm gathers more and more closely around the centers of the spore origins, and finally the latter break away from one another at all points of mutual contact (*fig. 13*), and the several independent protoplasmic masses round themselves off as eggs.

The reader will have noticed in the illustrations of this protoplasmic segmentation that each egg origin has a deeply stained center surrounded by delicate rays (*figs. 12-15*). These star-like structures are very conspicuous under low magnification (in *figs. 12* and *13*, 500 diameters), when the center appears to be a single structure. In reality it is not a simple unit, but is always composed of at least two structures, a coenocentrum accompanied by a nucleus. This dual nature is made clear only under high magnification, with clear preparations of very thin sections. I do not think it would be possible to understand the structure from entire mounts such as Hartog's. Hartog probably considered the center as a nucleus alone, and certain of Trow's figures indicate that he gave a similar interpretation. The coenocen-

trum is really the key to many of the problems of oogenesis in Saprolegnia.

The coenocentrum varies in its minute structure with different periods of oogenesis. It is at first a small body composed of several granules imbedded in dense material, from which a number of delicate fibrils radiate into the surrounding cytoplasm. The structure stains deeply and resembles an aster. After the eggs are fully formed the rays disappear and the coenocentrum grows larger, takes on a spherical form, and resembles a globule of oil or fat. It finally dissolves, sometimes with fragmentation, and completely disappears, in the older eggs. The coenocentrum is thus a structure peculiar to that period of oogenesis characterized by nuclear degeneration and the segmentation of the protoplasm to form the eggs. It bears a most important relation to these two events, which are the most difficult to study in the entire process of oogenesis.

We must begin with the first appearance of the coenocentra. These structures may always be found before the differentiation of the egg origins, at the time when the oogonium is filled with degenerating nuclei. The latter lie scattered through the cytoplasm (*figs. 14, 15*), and exhibit varying degrees of dissolution. The young coenocentra are always found in the densest regions of the protoplasm, portions destined to become egg origins, such as are shown in *figs. 14* and *15*. They are very small at first and would scarcely be noticed except for the radiating fibrils that mark their position. They increase in size as the egg origins take more definite form (*fig. 16*).

An examination of *figs. 14-16* shows at the side of each coenocentrum a small nucleus. This structure is very small at early periods of oogenesis (*figs. 14, 15*), and scarcely more clear than many of the degenerating nuclei in the neighborhood; but as oogenesis proceeds the nucleus accompanying the coenocentrum grows larger and increases greatly in staining material (*fig. 16*). When the eggs are fully formed, this nucleus is many times larger than at the first appearance of the coenocentrum, as may be seen by comparing *figs. 17-21* with *figs. 14* and *15*, which are all magnified 1,000 diameters. One would hardly think it

possible that the large nucleus present in the center of the mature egg was ever so small as the degenerating nuclei whose remains may be found in advanced stages of oogenesis (*fig. 16*), and sometimes even in the fully formed eggs (*figs. 17 and 23*); but there seems to be no doubt of this. The nucleus destined to preside over the egg is at first indistinguishable in size or structure from many of its neighbors. What should lead to its selection as the egg nucleus? I can see no other explanation than that its position gives it dynamic advantages, enabling it to survive when its neighbors lack the metabolic conditions necessary for nuclei and consequently must degenerate. This conceives the oogonium as too richly stocked with nuclei for the metabolic conditions of oogenesis, and in consequence the field of a struggle of the parts ("der Kampf der Theile," Roux).

What is the relation of the coenocentrum to these events? As we have stated, the coenocentrum is not a permanent organ either in the oogonium or the egg. It appears with the first indications of the egg origins and passes away as the eggs grow older. It is obviously a transitory structure peculiar to the most active periods of oogenesis. To the writer the coenocentrum seems to be the morphological expression of dynamic activities in the oogonium, and especially in the egg origins at the time when these are differentiated. It has the appearance of being the focal point in the center of the egg origins of the metabolic conditions peculiar to oogenesis; and this offers a very plausible explanation of the survival of the nucleus which lies nearest the coenocentrum.

The nucleus most fortunate in its position near the coenocentrum should be greatly benefited if this is a region of the protoplasm more favorably nourished than other parts. It is probable that the coenocentrum even draws toward itself nuclei within a certain sphere of attraction. Nuclei may be found with a pointed end extended toward the coenocentrum (*figs. 16, 20*). It will be remembered that Stevens (1901) showed with great clearness for *Albugo candida* and *A. Tragopogonis* that the nuclei in the immature eggs stretch toward the coenocentra so that their long dimensions are frequently twice the width. The nuclei of

Saprolegnia are too small to present conspicuous morphological evidence of this character; but we have the fact that the favored nucleus is almost always pressed against the coenocentrum which, together with the appearance of the nuclei and what we know of the events in Albugo, makes it quite certain that the coenocentrum exerts a chemotactic influence.

The changes that come over the egg as it matures are illustrated in *figs. 16-21*, which show the usual uninucleate condition of the egg. Binucleate and trinucleate eggs will be described in the following paragraphs. The two most important events of maturation are the increase in size of the nucleus and the gradual dissolution and final disappearance of the coenocentrum. The growth of the nucleus involves not only the extent of the space inclosed in the nuclear membrane (*figs. 17-21*), but also means a great increase in the amount of staining material, chromatic and nucleolar. The latter must be very many times greater in quantity in old eggs than at the beginning of oogenesis (compare *fig. 16* with *figs. 20* and *21*). The coenocentrum decreases in size until it becomes a very small globule (*fig. 20*), or it may split up into several granules, which soon become lost in an ill-defined mass of denser protoplasm. The coenocentrum finally disappears, and the contents of the egg then arrange themselves around a central vacuole, with the nucleus taking a peripheral position. This is the structure of the mature egg, and is illustrated in *fig. 21*.

We will now consider some conditions that have given rise to much discussion, namely, the binucleate and trinucleate eggs. They have been found by Humphrey, Hartog, and Trow, and the present study indicates that they may be expected in any member of the Saprolegniales. Trow attached much significance to them as evidence of sexuality, but his conclusions seem to the writer open to much criticism and will be taken up later. *Figs. 22-25* illustrate several conditions that show how easily an egg may become binucleate. Suppose two nuclei lie near enough to the coenocentrum to share about equally the advantages of position. Then it is not likely that either will give way to the other. Such conditions in a young egg are shown in *fig. 22*.

Fig. 25 also represents a pair of nuclei one above the other and both extended toward the coenocentrum, which was fast breaking down. *Fig. 24* is very interesting. In this instance the coenocentrum is the center of a mass of protoplasm considerably larger than the average egg. There are two well-developed nuclei, and the form of the cell suggests the probability that material which ordinarily would have gone into two egg origins has been held together in this instance by the influence of an especially large coenocentrum. An illustration of quite the reverse condition is shown in *fig. 23*, and is remarkable. Here we have presented an egg with two coenocentra, and at the side of each a nucleus. There is no doubt from the age of the eggs that the two nuclei in each of these cases are sister nuclei. It is plain that the processes that work for the segmentation of the protoplasm in the oogonium are complex, not all in the influence of the coenocentrum, nor yet all in the general activities of the cytoplasm.

Give the egg two nuclei with a fair start over their degenerating neighbors, and they seem to be able to exist side by side, not differing, so far as one may see, from the nuclei of uninucleate eggs. The two nuclei may lie far apart, as in *fig. 26*, or so near together that they touch, as in *figs. 25* and *27*; but in no instance—and I have seen a great many binucleate eggs—have I ever observed them fusing. Trow (1899) reported an instance of nuclear fusion in the egg, but the writer thinks we are justified in waiting for confirmation of this observation before attaching to it the importance given by that author.

Trinucleate eggs are somewhat rare in *Saprolegnia mixta*. I have seen hardly more than a dozen, and these were all rather mature examples. I have never been fortunate enough to find young stages, periods comparable to *figs. 22–24* of the binucleate eggs. The three nuclei may be grouped close together in the egg (*fig. 28*), or may lie quite separate from one another (*fig. 29*). There is no evidence that they fuse. The rather meager data at hand indicate that when there are three nuclei in an egg they are individually smaller than the single nucleus in an ordinary egg (compare *figs. 28* and *29* with *figs. 19–21*). This is to be

expected, for in general the three nuclei share between them the metabolic possibilities of about the same amount of protoplasm as is in the uninucleate egg. The trinucleate egg probably develops, as does the binucleate, from an egg origin in which more than one nucleus by fortunate position is able to survive the processes of general degeneration.

Let us now examine Trow's position respecting sexuality in the Saprolegniales. It is presented most completely in his 1899 paper. I approach this subject with some diffidence, for it has already been the occasion of detailed discussions of a personal character (Hartog, 1896, 1899). The matter is finally reducible to a question of confidence in Trow's evidence, his account, and his figures. Everyone must admit the possibility of sexuality in the Saprolegniales, but the question for us is, does Trow prove it?

The binucleate egg gave Trow the conviction, as he acknowledges, that fertilization took place through the introduction of a male nucleus into the egg from an antheridial tube. But the present studies show that binucleate eggs are quite common in an undoubted apogamous form, the material being entirely free from antheridial filaments. Moreover, these binucleate eggs have been followed through younger stages back almost to the period of the egg origins, and we know that these two nuclei were sisters in the oogonium. To make this point more plain, let the reader contrast the appearance of the two small nuclei shown in *figs. 22* and *23* with the nuclei in older eggs (*figs. 19-21*), and it will be evident that the former have the size and structure of nuclei in the young oogonium, and not of the fully mature gamete (egg) nucleus. It should also be noted that Hartog's binucleate eggs were also from apogamous material (Hartog, 1898 and 1899, p. 450).

If, then, apogamous material may have binucleate eggs, and the events of oogenesis explain the conditions, we are justified in examining Trow's evidence of sexuality very critically and demanding of it exceptional fulness and accuracy. We are concerned chiefly with Trow's figures, for they should show most exactly what the investigator really saw. I have been impressed

with the lack of detail in many of these figures, which has led me to think that Trow may have made a number of mistakes which would quite invalidate his evidence in support of sexuality. *Figs. 43, 44, and 46*, labeled "female gameto-nuclei," give appearances which are very similar to coenocentra, and I fear that he was not able to separate these structures in his preparations. *Fig. 35* certainly indicates that his material had coenocentra. But the most serious difficulties are encountered in his drawings of male gameto-nuclei (*figs. 45, 46*). These are not clear enough to be convincing; indeed, they seem to the writer to be the remains of degenerating nuclei at the periphery of the egg. Side by side with the structures labeled "male gameto-nuclei" Trow figures bodies, very similar in appearance, which are probably degenerate nuclei. In the face of this uncertainty and seeming contradiction of evidence the illustration of an antheridial filament piercing the egg (Trow, 1899, *fig. 45*) loses much of its weight, and the statement that two nuclei fuse in the center of the egg (Trow, 1899, *fig. 47*) is open to much doubt. The subject is so difficult that there are abundant opportunities for error, and we are justified in asking for much more evidence before accepting such important conclusions.

The writer cannot better sum up his attitude toward Trow's opinions on sexuality in the Saprolegniales than by defining them as *not proven* and *improbable* in the face of the mass of observations upon which botanists have generally agreed that the group is apogamous. The view of apogamy formerly resting entirely on the failure to find antheridial tubes fusing with the eggs is now supported by the present investigation on the details of oogenesis. These show that the binucleate egg, formerly difficult to understand on the theory of apogamy, may arise very naturally in a multinucleate oogonium when the method of oogenesis is as just described for *Saprolegnia mixta*.

The binucleate and trinucleate eggs of *Saprolegnia* are essentially similar to the multinucleate eggs of *Albugo Bliti* and *A. Portulacae*, and the conditions in the young eggs of *A. candida* and *A. Tragopogonis*, as described by Stevens (1899-1901). The latter, it will be remembered, contain several

potential gamete nuclei, but, so far as we know, only one of these becomes functional. But it would not be surprising to find at any time binucleate or trinucleate eggs among species of *Albugo* that are normally uninucleate.

In concluding, we must lay emphasis upon the importance of the coenocentrum as an index of the activities peculiar to oogenesis in *Albugo*, *Peronospora*, *Sclerospora*, *Pythium*, and *Saprolegnia*. Although this structure is probably in large part the expression of activities of the protoplasm as a whole, still there can be no doubt of its material existence. It is difficult to understand how Trow (1901, p. 291) can question this point, except that his figures indicate that fine details of structure were not shown in his preparations.

It would be strange, indeed, if so large a mass of protoplasm as the coenocentrum should not react in turn on the protoplasm that gave it birth. The coenocentrum is not a mass of food material, even though much of its granular substance may be the products of metabolism, and the structure as a whole trophoplasmic in character. It is protoplasm, and as such must be counted a factor in the subtle processes of oogenesis. Trow's comparison of the coenocentrum to a whirlpool in a river is not good, for there is unquestionably in this structure the expression of chemical phenomena as well as physical. The evidence is very strong from Stevens's (1901) work on *Albugo*, and the present study on *Saprolegnia*, that the coenocentrum has a sphere of chemotactic influence on the nuclei in its neighborhood.

SPOROGENESIS.

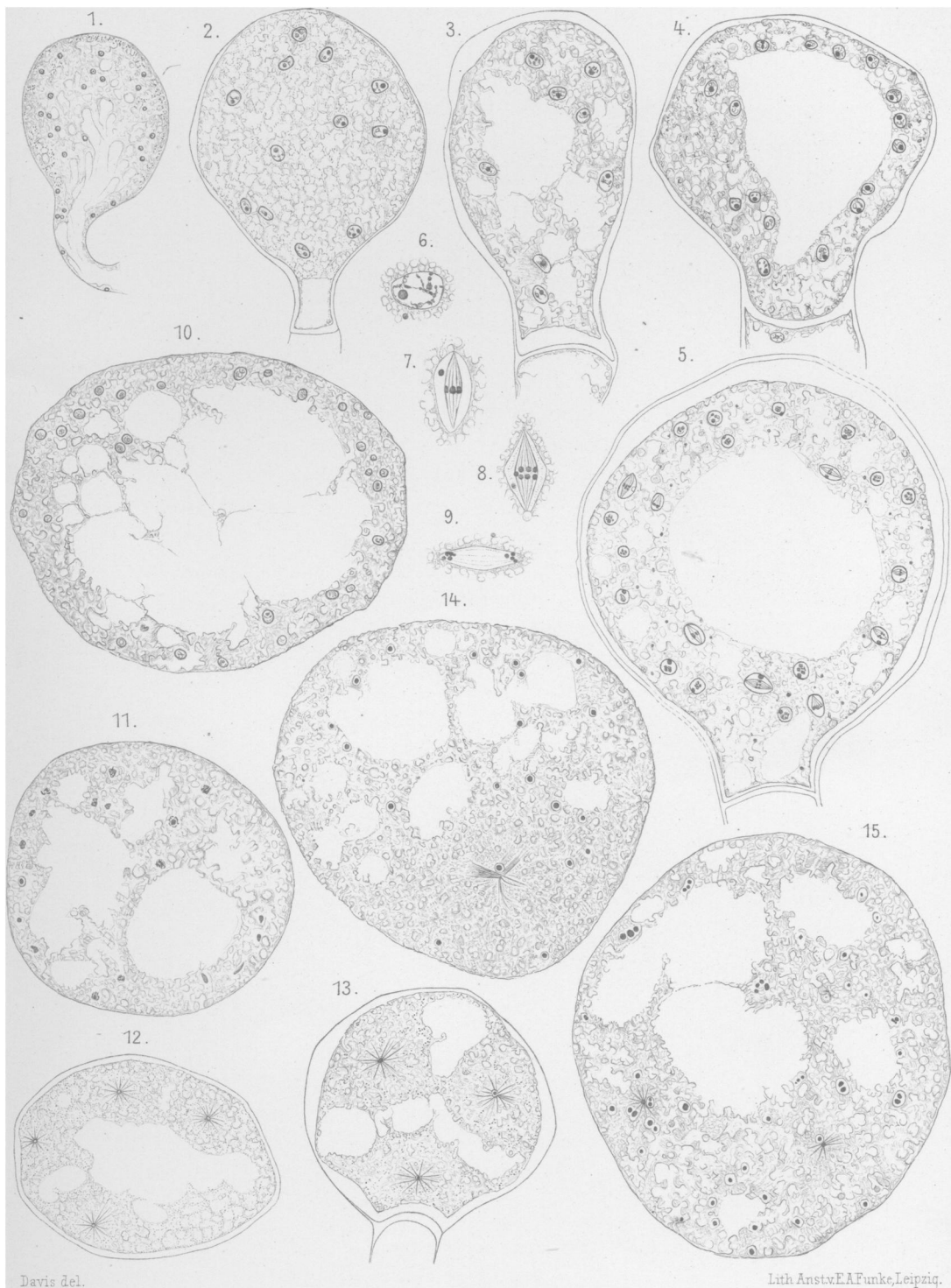
Except for a recent paper by Timberlake (1902) on *Hydrodictyon*, we know little of the details of zoospore formation in either algae or fungi, and the field would certainly repay investigation. The writer examined the sporangium of *Saprolegnia mixta* to contrast the conditions there with the processes of oogenesis, but, the subject not being favorable, little came of the study, except a general confirmation of the accounts of sporogenesis given by Rothert (1888), Hartog (1888), and Humphrey (1892). If the oogonium is the homologue of the sporan-

gium, we should expect a general similarity in the protoplasmic activities of each structure. There is the general agreement that the protoplasm segments by cleavage planes determined chiefly by vacuoles; but beyond this the activities of the two structures have little in common and a great many peculiarities.

As is well known, there is no mitosis in the sporangium. A large number of nuclei are carried into the tip of the hypha by the accumulation of protoplasm there. Vacuoles collect and develop in the center of the young sporangium (*fig. 30*), and flowing together form a large central space inclosed in a vacuolar membrane (*fig. 31*). The nuclei then lie scattered in the peripheral layer of protoplasm, and presently clefts appear which work outward between the nuclei from the central vacuole (*fig. 32*). The clefts divide the protoplasm so that it is cut up into polygonal areas, with clearer regions between. These are the zoospore origins, and each contains a nucleus.

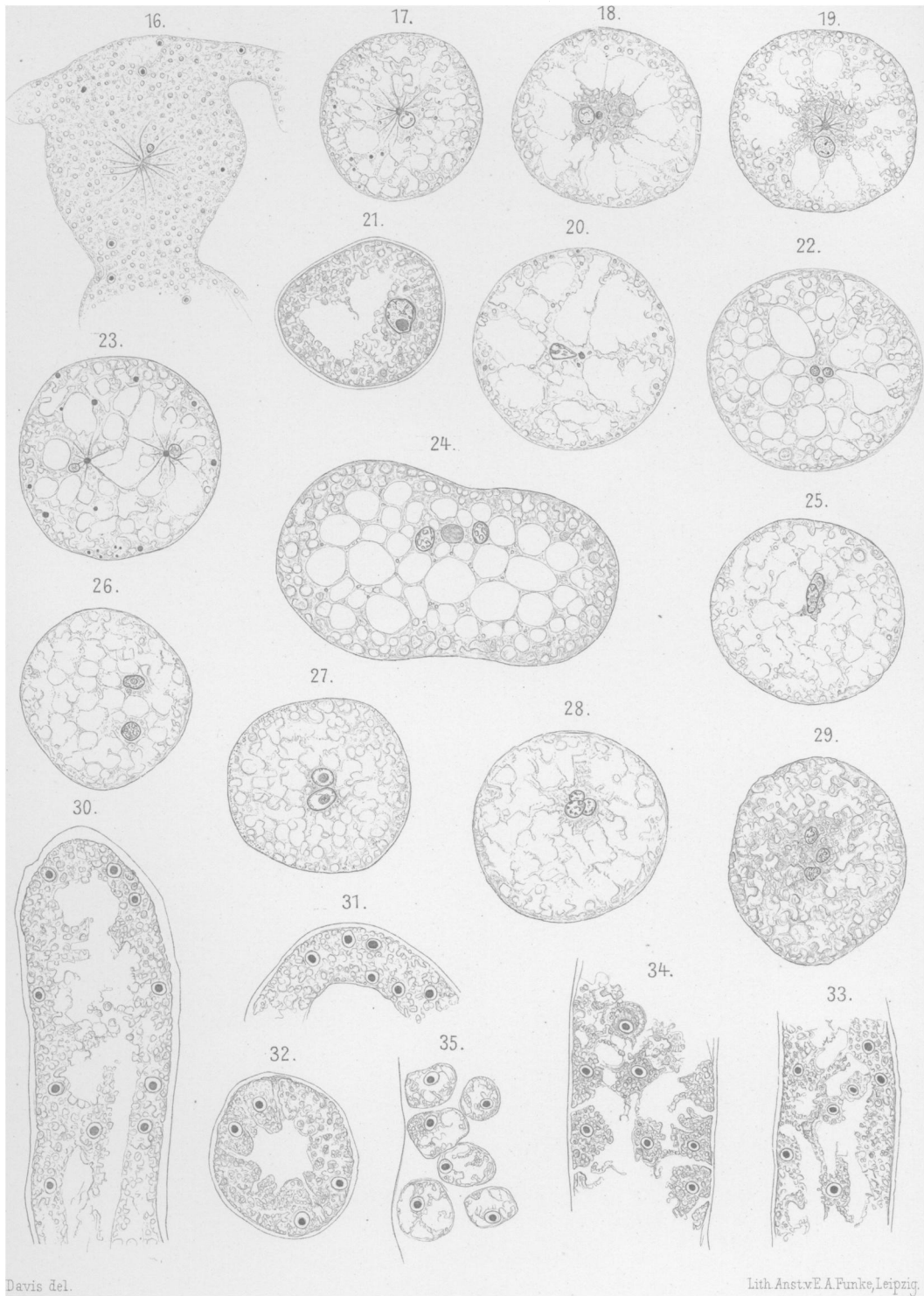
Rothert's explanations of succeeding conditions, which have also been confirmed by Humphrey and Hartog, seem entirely satisfactory. The sporangium is in a state of turgor when the clefts arise and push their way from the central vacuole toward the periphery. They finally reach the cell wall and immediately make possible the relief of the fluid in the central vacuole. There is at once a very evident decrease in turgor, which has an interesting effect on the appearance of the spore origins. The polygonal areas run together, and the whole sporangium becomes again almost homogeneous in structure. This means that the contraction of the sporangium brings the spore origins so close together that the clefts become almost obliterated. The spore origins also swell. They then begin to separate slowly, preliminary to their being finally rounded off as zoospores. There is a period when the small masses of protoplasm form a very irregular network through the sporangium (*fig. 33*), and this is followed by a more regular arrangement (*fig. 34*), in which the spore origins are connected by very delicate protoplasmic strands. The latter are finally broken and the bodies round off as zoospores.

The writer searched persistently in the sporangium for cyto-



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plasmic centers around which the process of segmentation might proceed, in the hope that light would be thrown upon the problem of the coenocentrum, but the examination brought forth no evidence of such structures in the sporangium. The nuclei themselves seem to be the ultimate centers of segmentation. The coenocentrum, therefore, so far as we know, is a structure peculiar to the oogonium.

[*To be concluded.*]